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A new species of *Litomosoides* (Nematoda: Onchocercidae), parasite of *Nectomys palmipes* (Rodentia: Cricetidae: Sigmodontinae) from Venezuela: description, molecular evidence, *Wolbachia pipientis* screening

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Abstract: The onchocercid filaria *Litomosoides taylori* sp. n. is described from the sigmodontine cricetid *Nectomys palmipes* Allen et Chapman in northeast Venezuela. A voucher specimen of the new species was used for molecular analysis of the *coxI* and 12S rDNA genes, and screened for the presence of the endobacterium *Wolbachia pipientis*. *Litomosoides taylori* belongs to the "*sigmodontis* group" of *Litomosoides* and a combination of characters can be used to distinguish it from the remaining 18 species forming this group. Among the five *Nectomys* species, all living near running water, *N. squamipes* also harbours *Litomosoides* species, *L. khonae* in Brazil and *L. navonae* in Argentina. These three *Litomosoides* species of the "*sigmodontis* group" do not share any particular characters. Gene sequences of *L. taylori* differ from those of the five *Litomosoides* species available, the three of the "*carinii* group" being the most distant. The new species harbours *W. pipientis,* which is concurrent with the great majority of *Litomosoides* species screened to date.

Keywords: *Litomosoides*, Onchocercidae, integrative taxonomy, *Wolbachia* screening

Species of the filarial genus *Litomosoides* Chandler, 1931 (Onchocercidae) are a component of the Neotropical fauna because they are common parasites of several groups of small mammals, marsupials, rodents and bats (Chandler 1931, Bain et al. 1980, Brant and Gardner 2000, Guerrero et al. 2002, Notarnicola et al. 2010). It has been emphasized that this "zoologically non-coherent" host-spectrum (*sensu* Chabaud 1982) is the signature of an evolution driven by host-switching (Bain et al. 1991, Brant and Gardner 2000). Experimentally, the macronyssid mite *Ornithonyssus bacoti* (Hirst, 1913) has been established as intermediate host for *Litomosoides* species, irrespective of the mammalian host group (Guerrero et al. 2006).

The greatest biodiversity of *Litomosoide*s is observed in parasites from phyllostomid bats and cricetid rodents of the Sigmodontinae. The species described herein is a parasite of the water rat, *Nectomys palmipes* Allen et Chapman. It was collected during the course of an environmental impact study conducted in the region of Los Llanos in Venezuela, which yielded approximately 175 filarioids from 550 small mammals (Guerrero 2008).

An integrated analysis, linking morphological and molecular techniques, was used to study the filarial sample as in previous works (Junker et al. 2009). Moreover, presence of the rickettsial endosymbiont *Wolbachia pipientis*, often harboured by onchocercid filariae, was investigated, knowing that this bacterium has been detected in four of the five species of *Litomosoides* screened till present (Casiraghi et al. 2004), and tissular *Wolbachia*-like bacteria were identified by electron microscopy in a sixth species (Cardenas and Lanfredi 2008).

MATERIALS AND METHODS

The study area belongs to the Eastern Llanos region, and is located south of Pariaguan in the state of Anzoategui, in the

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petroleum district called San Tome. It is a *Trachypogon* savannah, crossed by small rivers and with patches of gallery forest or palm tree (*Mauritia flexuosa*) forest. *Nectomys palmipes* were trapped in a palm tree forest, euthanized and checked for parasites under a stereomicroscope. Universal Transverse Mercator coordinate system is used for the geographical localisation.

Recovered parasites were fixed and preserved in hot 70% ethanol for morphological studies, or in absolute ethanol for DNA analysis (Ferri et al. 2009) and screening of the bacterial endosymbiont *Wolbachia pipientis* (Bain et al. 2008).

Specimens were cleared in lactophenol, measured and drawn using a microscope equipped with a camera lucida. Individual measurements are given for the holotype female and allotype male. Their respective descriptions are followed by measurements of paratypes given as the mean ± SEM. Measurement ranges are reported in Tables 2 and 3. Body length is given in millimetres; all other measurements are in micrometres. All specimens described were collected from a single host individual.

The morphological characters of the filariae were studied as described by Esslinger (1973) and Guerrero et al. (2002). The head was cut using a razor blade and observed in front view. Lateral chords and crests at vulva level were studied on transverse sections made at the level of vulva with a razor blade. The arrangement of male caudal papillae was observed on the tail in ventral view in several specimens; papillae are paired (papillae of a pair on a transverse line) or aligned (papillae of a pair more or less aligned on the ventral line). Two numbers are given for the *area rugosa*: the distance of the posterior end to cloaca, and the distance of the anterior end to cloaca. Host names used are consistent with Wilson and Reeder (2005) and Guerrero et al. (2011).

Parasites were deposited in the Parasitological Collection, Museo de Biologia, Universidad Central de Venezuela (Caracas) (CP-MBUCV), in the Muséum National d'Histoire Naturelle, Paris (MNHN), and a paratype in the Institute of Parasitology, BC ASCR, České Budějovice, Czech Republic.

For molecular analysis a female voucher specimen (MIB:zpl:00164) was transferred directly into absolute ethanol. DNA extract was prepared using the 5 PRIME, ArchivePure DNA Purification Kit. The filarial *coxI* and 12S rDNA gene sequences were generated following Folmer et al. (1994) and Casiraghi et al. (2004). The obtained sequences are about 600 and 500 bp long, respectively. PCR products were gel-purified using the 5PRIME, GelElute Extraction Kit and sequenced directly using ABI technology. All sequences have been deposited in the EMBL Data Library.

Sequences were checked visually with the BioEdit sequence alignment editor (version 7.0.5; Hall 1999), using GenBank nematode sequences as reference, and unambiguously aligned using Clustal X (Thompson et al. 1997). Nucleotide distances were calculated using MEGA 4.0 (Tamura et al. 2007) – options: nucleotide, Kimura 2-parameter, complete deletion, standard error computation by bootstrapping 500 replicates.

PCR screening for the presence of *W. pipientis* in the filaria was conducted following the methods described by Casiraghi et al. (2001, 2004), using general *Wolbachia* primers for 16S rDNA. PCRs were performed under different conditions (see Casiraghi et al. 2004) to increase screening sensitivity.

RESULTS

Litomosoides taylori Guerrero et Bain sp. n.

Figs. 1, 2

Description. Medium-sized *Litomosoides*, male one third of length of female; body tapering at both ends. Head attenuated, concave at mouth opening. Head papillae asymmetrically arranged: four externo-labial papillae (two dorsal and two ventral, close to sagittal plane) and two salient latero-ventral cephalic papillae; amphids not salient. Buccal capsule longer than broad (length/width 1.67–2.5), thinner in anterior part, posterior part embedded in oesophagus; external aspect of capsule wall not clearly delineated and irregular; in some specimens, a faint ring at mid-length present, more or less conspicuous depending on worm orientation (Fig. 1B, C). Buccal cavity conical anteriorly, tubular in posterior two thirds. Oesophagus apex most often dilated, followed by a constriction and a thickening just posteriorly; distinct division of oesophagus in muscular anterior and glandular posterior part, particularly in males. Nerve ring in posterior half of oesophagus. Tail long.

Female (Fig. 1): Post-oesophageal vulva and subspherical vagina. Lateral internal cuticular crest rounded in transverse section; lateral hypodermal chord narrow and thick. Tail long, thin, tip twisted and bent to left side.

Holotype: Total length 65.0, maximum width 250, at nerve ring 88, at oesophageal-intestinal junction 120. Buccal capsule 20 long and 10.5 wide; buccal capsule ratio 1.90. Nerve ring to anterior end 410. Oesophagus 620 long. Vulva 1540 to apex. Vagina 88 long by 80 wide. Tail 500 long, width at anus 70. Paratypes (n = 9): Total length 54.3 ± 1.94, maximum width 215 ± 8.1, at nerve ring 81 ± 7.2, at oesophageal-intestinal junction 106.6 ± 6.2. Buccal capsule 21.7 ± 1 long and 11 ± 0.5 wide; buccal capsule ratio 2.1 ± 0.14. Nerve ring to anterior end 421 ± 22.3. Oesophagus 606 ± 21.4 long. Vulva 1355 ± 63 to apex. Vagina 102 ± 2.1 long by 66 ± 2.9 wide. Tail 437 ± 28.5 long; width at anus 61 ± 4.2.

Microfilaria (from ovejector; n = 4): 58 (range 55–62) long and 3–4 wide; body attenuated at both extremities; salient cephalic hook; posterior extremity attenuated and pointed; last nucleus elongated, distant from tail tip. Sheath not identified.

Male (Fig. 2): Posterior region coiled; tail attenuated and finger-shaped, extremity rounded. Three to five pairs of caudal papillae, usually 4 pairs; papillae paired in anterior half of tail, more or less aligned in posterior half, the last reduced to an unpaired papilla; first pair adcloacal or slightly posterior; last papilla far from tail end. Left spicule with handle as long as lamina; lamina composed of a membranous folded ala and a terminal filament with rounded tip. Right spicule with slightly sclerotized terminal part composed of a non prominent dorsal heel, a distal pair of fine rods and a short membranous apical flap.

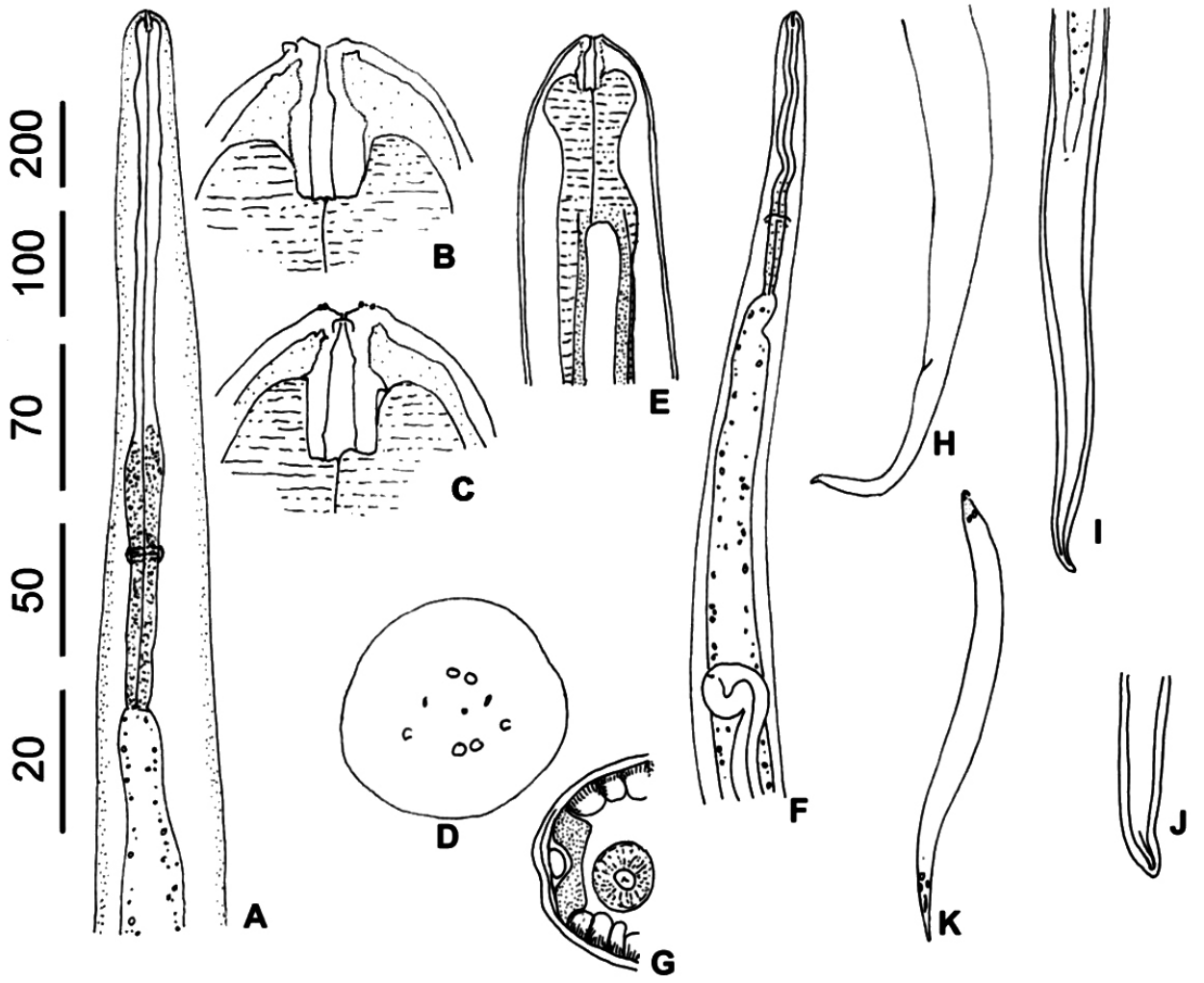

Fig. 1. *Litomosoides taylori* sp. n., female. **A –** oesophageal region, dorso-ventral view; **B, C –** head in ventral and lateral view, respectively (holotype); **D –** head in front view; **E –** cephalic region showing lateral internal crest and chord (holotype); **F –** anterior region, including vulva, subventral view; **G –** cross section at level of vagina (half the section is presented); **H –** posterior region, lateral view; **I –** tail, ventral view; **J –** twisted extremity of tail; **K –** uterine microfilaria. Scale bars: A, I = 100 μm; B–D, K = 20 μm; E, J = 50 μm; F, H = 200 μm; G = 70 μm.

Area rugosa composed of transverse bands of longitudinal crests.

Allotype: Total length 20.8, maximum width 140, at nerve ring 48, at oesophageal-intestinal junction 60. Buccal capsule 23 long and 10 wide; buccal capsule ratio 2.30. Nerve ring to anterior end 238. Oesophagus 580 long. Tail 179 long, width at anus 42. *Area rugosa* extends from 452 to 2132 from cloacal aperture. Left spicule 263 long, handle 130 long. Right spicule 73 long. Spicular ratio 3.36. Paratypes (n = 7): Total length 18.9 ± 0.7, maximum width 127 ± 5.3, at nerve ring 54 ± 2.2, at oesophageal-intestinal junction 64 ± 1.8. Buccal capsule 20.2 ± 0.4 long and 9.4 ± 0.4 wide; buccal capsule ratio 2.16 ± 0.07. Nerve ring to anterior end 273 ± 12.8. Oesophagus 533 ± 18.5 long. Tail 171 ± 3.3 long, width at cloaca 41 ± 1.1. *Area rugosa* 433 ± 29 to 1963 ± 110 from cloacal aperture. Left spicule 257 ± 6.2 long, handle 124 ± 2.1 long. Right spicule 81 ± 2.3 long. Spicular ratio 3.18 ± 0.1.

Type host: *Nectomys palmipes* Allen et Chapman, 1892 (Rodentia: Cricetidae: Sigmodontinae).

Type locality: Rio Castillito, 170 metres altitude, Municipio Jose Gregorio Monagas, Edo. Anzoategui, Venezuela. Geographic coordinates E20-310658 N957193.

Site of infection: Peritoneal and pleural cavities.

Prevalence and intensity: Three hosts examined, all infected; 29, 6 and 59 filariae per host.

Type material: Holotype female CP-MBUCV No. 6124; allotype male CP-MBUCV No. 6125; 3 male and 4 female paratypes CP-MBUCV No. 4816; 4 male and 4 female paratypes in MNHN Paris collection, accession number 44 YU. A female paratype in the Institute of Parasitology, BC ASCR, České Budějovice, Czech Republic (N-964). EMBL Data Library accession number: FR719324 for *coxI* and FR719325 for 12S rDNA.

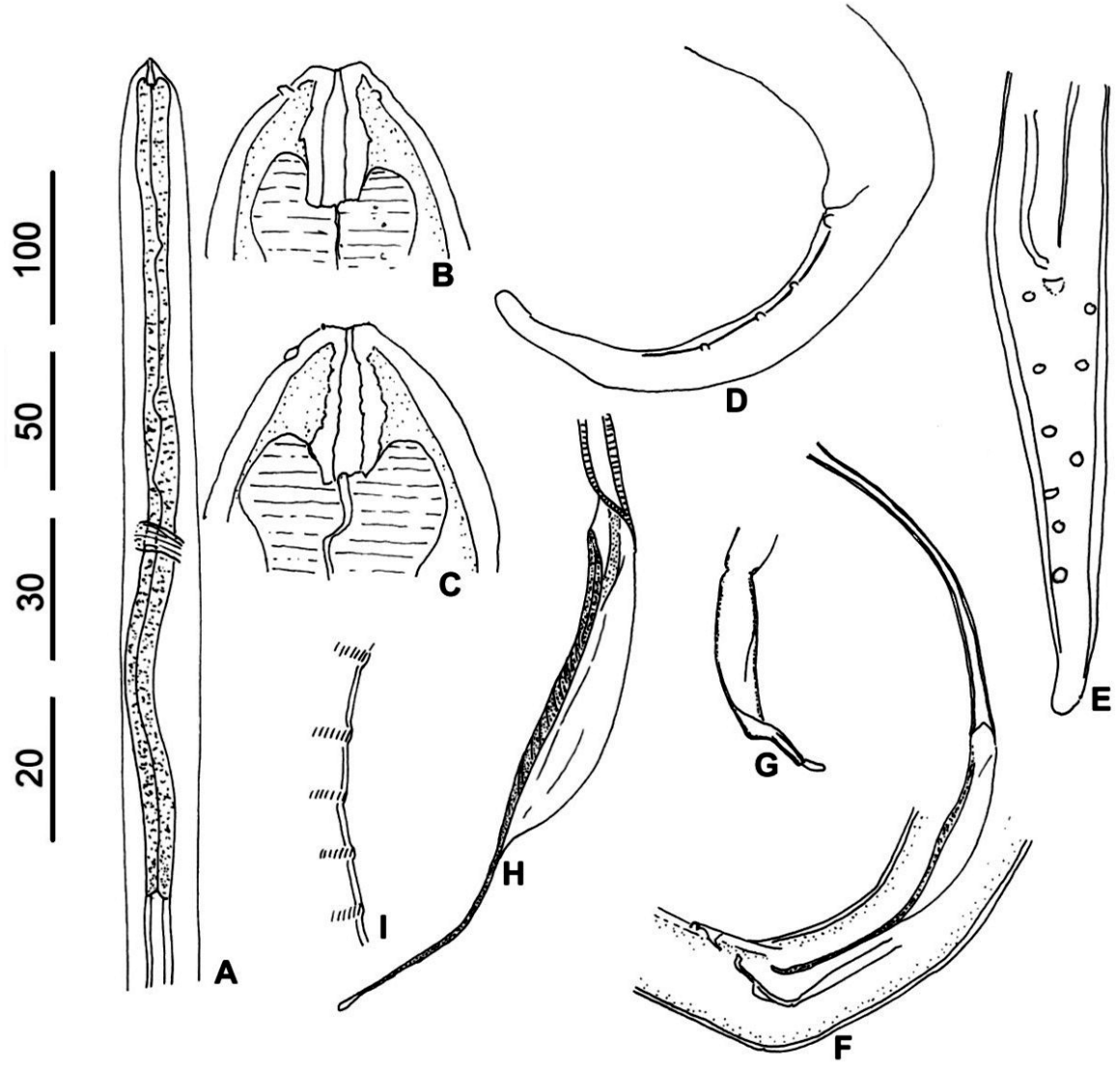

Fig. 2. *Litomosoides taylori* sp. n., male. **A –** anterior extremity, right lateral view; **B –** buccal capsule, ventral view; **C –** buccal capsule of another specimen, left lateral view; **D –** tail, left lateral view (allotype); **E –** tail, ventral view (allotype); **F –** spicules, left lateral view (allotype); **G –** distal end of right spicule, right lateral view; **H –** lamina of left spicule, left lateral view; **I –** detail of *area rugosa* at its mid-length, right lateral view (partial representation to show longitudinal crests and distance between transverse ridges). Scal bars: A = 100 μm; B, C = 20 μm; D–F 50 μm; G–I = 30 μm.

Etymology: The species is dedicated to Prof. David Taylor, University of Edinburg, in recognition of his dynamic collaboration during twenty years as coordinator of research programmes using *Litomosoides sigmodontis* as a model for filariases.

Remarks. Two morphological groups are identified in *Litomosoides*, the "*carinii*" and the "*sigmodontis*" groups (Bain et al. 1989). The new species belongs to the latter, based on the shape of its spicules (lamina of the left spicule with large membranous part; distal end of the right spicule not heavily sclerotized, with two rods and membranous tip). The "*sigmodontis* group" comprises 18 species parasitic in rodents, marsupials and bats (Table 1), all of which differ from *L. taylori* (Tables 2, 3). Their distinctive characters are outlined below.

Twelve species are parasites of Sigmodontinae: *Litomosoides patersoni* (Mazza, 1928), has shorter, stout microfilariae (35–44 long and 6 wide), a pair of precloacal papillae and its remaining caudal papillae are symmetrically arranged; it also has a complete set of head papillae and a straight female tail (Mazza 1928, Notarnicola et al. 2010). Five species have prominent amphids (Notarnicola et al. 2002, Bain et al. 1980, Notarnicola et al. 2000, Notarnicola and Navone, 2009, Notarnicola 2005). Microfilariae of three of these species are longer than 75 (55–62 in *L. taylori*); in addition, in *L. anguyai* Notarnicola, Bain

Table 1. Species of *Litomosoides* of the "*sigmodontis* group" and the five species known only by female or microfilariae.

Species	Host		Type locality
	Type species	Family	
L. anguyai Notarnicola, Bain et Navone, 2002	*Oxymycterus misionalis*	Cricetidae	Argentina
L. barretti Muller, 1980	*Micoureus demararae**	Didelphidae	Brazil
L. chagasfilhoi Moraes Neto, Lanfredi et De Souza, 1997	*Akodon cursor*	Cricetidae	Brazil
L. circularis (Linstow, 1899)	*Holochilus brasiliensis***	Cricetidae	Brazil
L. ctenomyos Brant et Gardner, 1997	*Ctenomys opimus*	Ctenomyidae	Bolivia
L. esslingeri Bain, Petit et Diagne, 1989	*Melanomys caliginosus*	Cricetidae	Colombia
L. fosteri Caballero, 1947	*Glossophaga soricina*	Phyllostomidae	Panama
L. galizai Bain, Petit et Diagne, 1989	*Oecomys trinitatis tapajinus*	Cricetidae	Brazil
L. hoplomyis Esslinger, 1973	*Hoplomys gymnurus*	Echimyidae	Colombia
L. kohnae Bain, Petit et Diagne, 1989	***Nectomys squamipes***	Cricetidae	Brazil
L. legerae Bain, Petit et Berteaux, 1980	*Oxymycterus quaestor*	Cricetidae	Brazil
L. leonilavazquezae Caballero, 1939	*Macrotus waterhousii*	Phyllostomidae	Mexico
L. nasuti Notarnicola et Navone, 2009	*Oxymycterus nasutus*	Cricetidae	Uruguay
L. navonae Notarnicola, 2005	***Nectomys squamipes***	Cricetidae	Argentina
L. oxymycteri Notarnicola, Bain et Navone, 2000	*Oxymycterus rufus*	Cricetidae	Argentina
L. patersoni (Mazza, 1928)	*Holochilus chacarius*	Cricetidae	Argentina
L. sigmodontis Chandler, 1931	*Sigmodon hispidus*	Cricetidae	USA
***L. taylori* Guerrero et Bain sp. n.**	***Nectomys palmipes***	Cricetidae	Venezuela
L. teshi Esslinger, 1973	*Carollia perspicillata*	Phyllostomidae	Colombia
Male unknown			
L. artibei Esslinger, 1973	*Artibeus jamaicensis*	Phyllostomidae	Colombia
L. chitwoodi Bain, Guerrero et Rodriguez, 2003	*Artibeus jamaicensis*	Phyllostomidae	Mexico
L. solarii Guerrero, Martin, Gardner et Bain, 2002	*Trachops cirrhosus*	Phyllostomidae	Peru
L. caliensis Esslinger, 1973	*Sturnira lilium*	Phyllostomidae	Colombia
L. colombiensis Esslinger, 1973	*Platyrrhinus dorsalis*	Phyllostomidae	Colombia

Species of *Nectomys* are in bold characters; *in Guerrero et al. 2002; **in Guerrero et al. 2011.

et Navone, 2002 the male has a pair of precloacal papillae; *L. legerae* Bain, Petit et Berteaux, 1980 has a complete set of head papillae (4 externo-labial and 4 cephalic), buccal capsule walls with alternatingly expanded and constricted parts, and a microfilaria with a caudal filament; in *L. oxymycteri* Notarnicola, Bain et Navone, 2000 the fourth pair of caudal papillae is joined on the midventral line and the oesophagus is undivided. Of the two remaining species with prominent amphids, *L. nasuti* Notarnicola et Navone, 2009 has no apparent cephalic papillae, its buccal capsule has a conspicuous ring and the female tail carries phasmidial knobs; *L. navonae* Notarnicola, 2005 possesses one cephalic papilla and the male tail is longer, with six or seven pairs of caudal papillae, several of which are asymmetrically arranged. Among the five species with non-salient amphids (Bain et al. 1989, Moraes-Neto et al. 1997), the buccal capsule wall of *L. galizai* Bain, Petit et Diagne, 1989 is thinner in both sexes as well as longer in the male (25–30 vs. 19–22 in *L. taylori*); *L. khonae* Bain, Petit et Diagne, 1989 is a longer species with shorter microfilariae, the tail is pointed in both sexes and curved dorsally in the female; in *L. chagasfilhoi* Moraes-Neto, Lanfredi et de Sousa, 1997, the buccal capsule wall is thinner, the oesophagus undivided and the left spicule has a long membranous terminal sheath; *L. sigmodontis* Chandler, 1931, redescribed by Bain et al. (1989), has longer microfilariae (84.5 ± 2.9) and a buccal capsule with thicker, irregular and well sclerotized walls. *Litomosoides esslingeri* Bain, Petit et Diagne, 1989 has an irregular buccal capsule wall, a cylindrical buccal cavity, a straight tail with a conical extremity in the female, large and flat lateral chords, and microfilariae with a large sheath. *Litomosoides circularis* (von Linstow, 1899) has a shorter buccal capsule in the males, a pair of adcloacal papillae, the fourth pair is joined on the midventral line, and the terminal third of the left spicule is rod-like with a bevelled extremity (von Linstow 1899, Guerrero and Bain 2011). Two species are parasites of rodents other than Sigmodontinae (Esslinger 1973, Brant and Gardner 1997): *Litomosoides hoplomyis* Esslinger, 1973 from Echimyidae is a very small species (males 10–13 and females 18–30 long) and its buccal capsule wall is thinner but with a thick anterior part. In *L. ctenomyos* Brant et Gardner, 1997 from Ctenomyidae, the oesophagus is longer and undivided in the female, and caudal papillae are symmetrically arranged along the male tail right to the tip.

The single species from marsupials, *L. barretti* Muller, 1980, is larger, with a shorter buccal capsule; its caudal papillae are all paired and no cephalic papillae have been described (Muller 1980).

Three species are parasites of bats (Caballero 1939, 1947, Esslinger 1973): *Litomosoides leonilavazquezae* Caballero, 1939, of which the description is not very precise, has a shorter buccal capsule and a finger-shaped

Table 2. Measurements of females of *Litomosoides* species of the "*sigmodontis* group" and the five species known only by female or microfilariae (body length in millimetres, other measurements in micrometres).

Group	Species	Total length	Buccal capsule		Oesophagus length	Vulva to apex	Tail length	Microfilariae	
			length	width				in uterus	in blood
"sigmodontis"	*anguyai**	48.7–74.0	17–24	6–9	624–975	1093–1820	272–767	80–112	–
	*barretti**	67.0–85.0	16[f]	–	670–800	1300–1800	400–530	–	60–73
	*chagasfilhoi**	86.9–95.0	18–25	3–4	600–775	1300–2257	375–600	61–92	62–72
	*circularis**	65.0	–	–	620	1300	690	–	–
	circularis[1]	59.9–80.8	15–20	8–9	546–667	1098–1806	544–633	62–63	–
	*ctenomyos**	–	17–20	6–11	833–872	1125–2170	593[f]	72	–
	*esslingeri**	56.0–104.0	20–24	9–13	384–656	840–2180	414–710	–	65–77
	*fosteri**	42.6	19	4	631	1460	213	–	–
	*galizai**	59.0–81.0	20–25	–	540–800	1400–2130	410–920	52–75	72–102
	*hoplomyis**	18.0–30.0	16–21	5–8	572–794	750–1120	233–382	–	58–71
	*khonae**	110.0–115.0	16–18	–	720–730	1920–2350	580	55–62	–
	*legerae**	80.0–97.0	21–24	–	1040–1240	1340–2100	415–555	80–83	45[f]
	*leonilavazquezae**	40.0–50.0	16	6	516–528	840–1300	468	–	52–56
	*nasuti**	37.4–47.9	16–22	7–10	450–760	850–1300	215–540	69–76	–
	*navonae**	42.8–77.1	18–25	8–11	580–1125	1300–2300	375–908	62–85	65–72
	*oxymycteri**	43.0–70.8	17–24	7–11	519–1023	1071–1650	293–590	75–103	–
	*patersoni**	61.0–92.0	–	–	900	1100	910[f]	–	35–46
	patersoni[2]	–	24–27	9–10	650–780	1350–2050	500	35–44	–
	*sigmodontis**	50.0–65.0	25[f]	–	650[f]	1250	625	100–105	–
	sigmodontis[3]	68.0	18–21	–	690–720	1100–1390	270–660	84.5	71–75
	***taylori* sp. n.**	43.0–65.0	18–25	9–13	520–680	1150–1595	330–535	55–62	–
	*teshi**	84.0–92.0	20–25	9–11	570–656	950–1420	296–445	–	75–109
	teshi[4]	89.0–91.0	20–22	–	640–650	960–1350	360–430	–	–
Male unknown	*artibei**	> 4.0	34	5	525	825	–	–	97–108
	artibei[4]	–	33–34	–	524–527	820–832	120–130	–	–
	*chitwoodi**	15.0	12	–	583	810	583	–	–
	*solarii**	52.9	21	7	935	910	250	50–60	–
	*caliensis**	–	–	–	–	–	–	–	53–65
	*colombiensis**	–	–	–	–	–	–	–	100–125

*from original author (see Table 1); [f] measured from original figure; [1] Guerrero and Bain 2011; [2] Notarnicola et al. 2010; [3] Bain et al. 1989; [4] Cuartas-Calle and Muñoz-Arango 1999.

tail in the female, the males have a shorter tail. *Litomosoides fosteri* Caballero, 1947 has a buccal capsule with two thickened rings, a shorter tail in both sexes, a shorter right spicule, eight pairs of caudal papillae and a higher spicular ratio (4.8 compared to 2.93–3.75 in *L. taylori*). *Litomosoides teshi* Esslinger, 1973 is a larger species with an asymmetrical buccal capsule and longer microfilariae (75–109 vs. 55–62 in *L. taylori*).

The males of the remaining six species are not known and, therefore, they cannot be assigned to the "*sigmodontis*" or "*carinii*" group. Of three of these the females are known (Esslinger 1973, Guerrero et al. 2002, Bain et al. 2003): *Litomosoides artibei* Esslinger, 1973 has a longer buccal capsule (34); *L. solarii* Guerrero, Martin, Gardner et Bain, 2002 has a tubular buccal cavity with thin capsule walls, a longer oesophagus, the vulva is situated in the oesophageal region and the microfilariae are cylindrical, with a posterior extremity that abruptly attenuates to a sharp point; *L. chitwoodi* Bain, Guerrero et Rodriguez, 2003 has a shorter buccal capsule (12). Of the remaining two species, only the microfilariae are known: *L. colombiensis* Esslinger, 1973 is twice as long and tapers posteriorly to form a narrow tail tip; *L. caliensis* Esslinger, 1973 has a thicker tail with a rounded extremity (Esslinger 1973).

Molecular analyses

Using an integrated taxonomical approach based both on morphological characterisation and DNA barcoding (Ferri et al. 2009), the new species was also characterised by 12S rDNA and *coxI* sequence analysis. The datasets used for this work comprise a total of 188 sequences for *coxI* and 199 sequences for 12S rDNA. The more closely related nematodes were detected within the genus *Litomosoides* itself. Among the five *Litomosoides* species analysed to date (Casiraghi et al. 2004), three belong to the "*carinii* group", *L. brasiliensis* Almeida, 1936, *L. hamletti* Sandground, 1934 and *L. yutajensis* Guerrero, Martin et Bain, 2003, and are more distant to the new species than those of the "*sigmodontis* group", *L. sigmodontis* and *L. galizai. Litomosoides galizai* (EMBL Data Library accession numbers: 12SrDNA AJ544849; *coxI* AJ544870), a parasite of *Oecomys* (see Table 1), is closest to the new species: K2P nucleotide diversity was 5.4% (standard de-

Table 3. Measurements of males of *Litomosoides* species of the "*sigmodontis* group" (body length in millimetres, other measurements in micrometres).

Species	Total length	Buccal capsule		Oesophagus length	Left spicule	Right spicule	Pairs of cloacal papillae[a]	Tail length
		length	width					
*anguyai**	19.1–27.4	15–21	6–8	456–815	240–370	64–91	1-1-3/4	174–214
*barretti**	22.0–24.4	–	–	470–660	333	100–115	0-1-5	230
*chagasfilhoi**	21.9–30.0	17–22	3–4	513–587	296–316	81–94	0-1-4/6	144–188
*circularis**	20.0	–	–	440	260	190	0-0-7	160
circularis [1]	21.5–24.6	14–16	8	454–556	250–311	98–121	0-1-3/4	119–198
*ctenomyos**	27.0–31.0	13–18	7 – 9	511–730	189–385	73–91	0-1-8/9	151–194
*esslingeri**	15.0–25.0	16–23	7–11	380–583	255–315	80–106	0-1-3/5	151–200
*fosteri**	20.8–21.6	15–19	4	646	274	57	0-0-8	87–99
*galizai**	17.0–19.2	25–30	–	610–640	300–345	78–82	0-1-2&4	242–250
*hoplomyis**	10.0–13.0	15–19	5–7	604–705	180–238	53–77	0-0-3/4	135–171
*khonae**	22.2–22.7	15–17	–	550–680	285–305	75–92	0-1-3/4&5/6	225–280
*legerae**	25.0–27.0	22	–	720–800	300–360	85–90	0-1-3	200–248
*leonilavazquezae**	19.3–19.5	18–20	4	429	209–229	82–86	0-1-3	73–106
*nasuti**	16.3–17.9	15–16	7–8	540–600	285–300	70–75	0-1-3	170–190
*navonae**	18.1–28.0	16–21	6–9	432–750	220–375	80–95	0-1-5/6	210–280
*oxymycteri**	15.6–23.6	18–23	7–11	532–980	231–340	80–167	1 unpaired-1-4	186–240
*patersoni**	20.0–22.0	–	–	550	125	50	0-1-2	250
patersoni [2]	15.8–19.3	19–22	7–8	510–580	246–247	80–87	1-1-3/4	146–275
*sigmodontis**	24.0–28.0	20	–		185–295	75–80	0-0-4	180
sigmodontis [3]	18.1–20.1	17	–	540–580	255–295	78–90	0-1-3&5	165–170
***taylori* sp. n.**	16.3–20.8	19–22	8–11	460–600	235–285	71–88	0-1-3/4	158–184
*teshi**	21.0–27.0	17–24	8–14	393–737	255–329	70–90	0/1-4/5	154–206

[a] precloacal-adcloacal-postcloacal (n/n' when asymmetrically arranged); *from original author (see Table 1); [1]Guerrero and Bain 2011; [2]Notarnicola et al. 2010; [3]Bain et al. 1989.

viation 1.3%) for 12S rDNA and 12.6% (standard deviation 1.5%) for *coxI*.

PCR screening for *Wolbachia* was positive.

DISCUSSION

Nectomys spp. are linked with running water in South America. The five Neotropical species have distinct geographic areas (Wilson and Reeder 2005): *N. squamipes* (Brants) occurs in the southeast, *N. apicalis* Peters in the west, *N. rattus* (Pelzen) in the Amazonian region, and the two remaining species are restricted to the northern areas, with *N. magdalenae* (Thomas) in the west, and *N. palmipes* in the east. *Litomosoides* is at present reported in three *Nectomys* species. *Nectomys squamipes* is infected with *L. kohnae* in Brazil (Sao Paulo) and with *L. navonae* in Northern Argentina (Misiones, type locality; Chaco, Formosa). *Nectomys palmipes* is infected with L. *taylori* in northeast Venezuela. The *Litomosoides* from *Nectomys* spp. belong to the *sigmodontis* type. No distinctive shared characters indicating that they form a particular lineage could be observed among these three *Litomosoides* species from the same host genus. The molecular analysis indicates *L. galizai*, from the sigmodontine *Oecomys trinitatis tapajinus* Thomas from Parà, Brazil, as the closest species. However, it must be stressed that species in the "*sigmodontis* group" are very closely related, and gene sequencing in combination with morphological studies is necessary to decipher the diversity and evolution of this filarial genus. Presently, this has been achieved for a few species only, not including the parasites of *N. squamipes*.

Regarding the endosymbiont *Wolbachia pipientis*, its presence was expected, since among the seven *Litomosoides* species examined to date, *L. yutajensis* from a mormoopid bat was the only one not to harbour this bacterium (Bain et al. 2008).

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